normal neutral sphingomyelinase activity (Table 1). SM deacylase activity could not be observed (not shown).

AD sample no. 4 emerged as a special specimen. We never found free fatty acids to be generated from PC in other skin samples, but surprisingly, chronic dermatitis sample no. 4 hydrolyzed C_5-PC (Figure 1). When 1,2-bis C_{11}-PC in which both of the esterified fatty acids are labelled was used as the substrate, C_{11}-free fatty acid was detected (Figure 2). In contrast to all of the other AD specimens, sample no. 4 generated some products detectable between the plate origin and 1,2-bis C_{11}-PC. These bands might correspond to C11-SM or to lyso-PC. The faint band intensities, however, point to rather low amounts as products of 1,2-bis C_{11}-PC containing only one labelled fatty acid reveal increased emission intensities as compared to 1,2-bis C_{11}-PC in which the adjacent fluorophores quench themselves. The more intense emission of the free fatty acid might indicate that both of the acyl-chains were hydrolyzed but mostly not re-esterified (Figure 2).

Missing transacylase in a normal skin specimen indicates that the loss of this enzymatic activity is not sufficient for AD development. On the other hand, one of the AD samples (no. 4) might contain some transacylase activity that does not protect against AD. Conversely interpreted, the one exceptional normal individual might be at risk for AD development, and the special AD patient might await remission of disease.

In conclusion, we found that AD skin may be associated with a lack of PC-SM transacylase activity.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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The Detrimental Effects of Daily Sub-Erythemal Exposure on Human Skin *In Vivo* Can be Prevented by a Daily-Care Broad-Spectrum Sunscreen

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TO THE EDITOR

Skin cancer results from chronic solar exposure, especially in fair-skinned people (Armstrong *et al.*, 1997). The International Agency for Research on Cancer (IARC) assessed the role of sunscreens in skin cancer prevention and concluded that ''There is limited evidence in humans for a cancer preventive effect of the topical use of sunscreen formulations against squamous-cell carcinoma of the skin'' (IARC, 2001). Furthermore, there was ''inadequate evidence'' for any preventive effects against basal cell carcinoma and malignant melanoma.

Photocarcinogenesis involves DNA photodamage, inadequate DNA repair, probable failure of apoptosis, mutation (Wikonkal and Brash, 1999), clonal expansion of mutated cells (Brash *et al.*, 2005), and UVR-induced immunosuppression (Ullrich, 2002; Ullrich *et al.*, 2002). Most human photobiology research is with a single erythemal exposure that is not representative of the effects of repeated sub-erythemal exposure, which may initiate adaptive responses such as tanning and DNA repair (Sheehan *et al.*, 1998, 2002; Agar

Abbreviations: CPD, cyclobutane pyrimidine dimer; LC, Langerhans cell; MED, minimal erythema dose; SBC, sunburn cell; SSR, solar simulating radiation; TT, thymine dimer

and Young, 2005). Many earlier repeat exposure studies were performed with non-solar spectra, but more recent work has been performed with solar simulating radiation (SSR) (Seite *et al.*, 2006).

We studied the effects of 11 consecutive daily sub-erythemal exposures of SSR on buttock skin of six healthy sun sensitive skin types 1/II (20–30 years) (Materials and Methods and Table S1). A standard dose was given on each exposure that represented 0.52 or 0.65 minimal erythema doses (MED) depending on the MED of the volunteer. We assessed erythema daily and, on days 5, 11, and 12 (24 hours after last exposure), end points relevant in skin cancer, including thymine dimers (TT), p53 and Bcl2 protein expression that regulates apoptosis, apoptotic sunburn cells (SBC), Langerhans cell (LC) depletion, and epidermal turnover. Standard quantitative histological and immunohistochemical techniques were used and are described in more detail in the Supplementary Material (Materials and Methods and Table S2). We also evaluated the effects of a broad-spectrum (4* UVA) daily-care low sun protection factor (7.5) sunscreen (2 mg/cm^2) with 6% polysilicone-15 (Parsol SLX) as a UVB filter and 2% butyl methoxydibenzoylmethane (Parsol 1789) as a UVA filter (see Figure S1 in Materials and Methods for absorption profile). The study, approved by the Ethics Committee of St Thomas' Hospital London, was performed according to the Declaration of Helsinki Principles and volunteers gave written informed consent.

EFFECTS OF REPEATED SUB-ERYTHEMAL SSR AND MODIFICATION BY THE SUNSCREEN

Erythema accumulated on the vehicle control sites but not on the sunscreen sites (Figure 1a). At day 12, the vehicle site showed a florid erythema and considerably more DNA damage than the sunscreen site (Figure 1b).

Overall, sunscreen-treated sites showed much less DNA damage (TT) than the vehicle sites at all time points ($P<0.01$–0.03) (Figure 2a). P53 expression was very low on all sunscreen-treated sites (Figure 2b). Much higher expression was seen on the vehicle sites but with high inter-personal variation. Significant protection ($P<0.01$) was only seen at day 11. There was no difference in Bcl2 expression between the 0 MED control and any other site (data not shown) and no SBC were seen on any site. There was a time-dependent trend for depletion of LC with the vehicle that was significantly different from 0 MED at days 11 ($P=0.02$) and 12 ($P<0.01$) (Figure 2c). The sunscreen sites were not significantly different from the 0 MED control at any time point. Sunscreen protection, compared with vehicle, was significant at days 5 ($P=0.04$) and 12 ($P<0.01$). There was a high inter-personal variation in an immunomarker of epidermal turnover (MIB1/Ki-67), even with the 0 MED control site (data not shown). Sunscreen values were lower but not significantly so. The mean number of viable cell layers was not affected (data not shown) by any treatment. Our data can be compared with another study (Seite *et al.*, 2006) that assessed the effects of nine daily exposures of 0.5 and 0.75 MED SSR (but with a lower UVB content than our spectrum). This work also showed increased p53 expression and LC depletion. The authors reported SBC induction, but this was only up to 1.5 SBC/cm that is unlikely to be biologically significant.

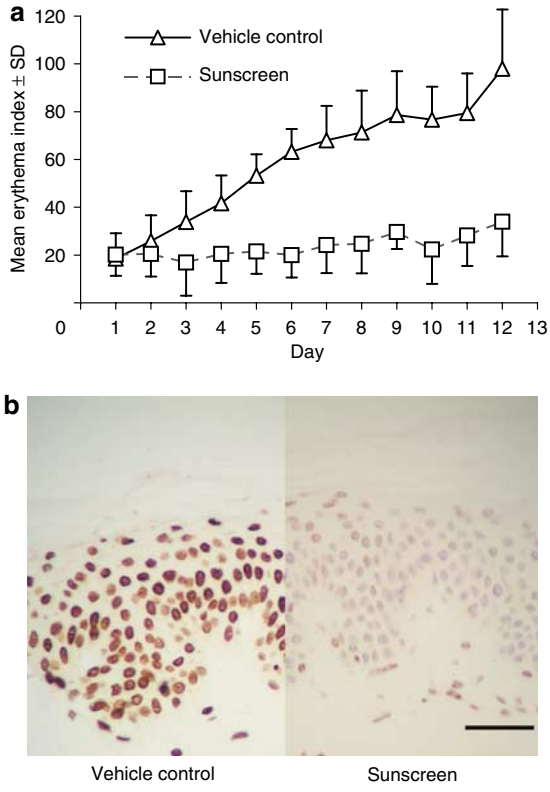

Figure 1. The accumulation of clinical and molecular photodamage is prevented by the daily use of a sunscreen. (**a**) Erythema, assessed by reflectance spectroscopy, after daily sub-erythemal exposure is prevented by sunscreen application. Note that the eye starts to detect erythema at an erythema index (EI) of about 50, (**b**) TT on day 12 from one volunteer, 24 hours after final irradiation showing vehicle control and sunscreen-treated skin. Bar = 50 μm.

POSSIBLE ROLE OF SUNSCREENS IN THE PREVENTION OF CHRONIC PHOTODAMAGE

Daily sub-erythemal SSR causes significant clinical, cellular, and molecular damage in skin types I/II and that can be inhibited by the use of a broad-spectrum daily-care sunscreen with a low sun protection factor and 4* UVA protection.

The cyclobutane pyrimidine dimer (CPD), of which TT is one type, is important for skin cancer (Jans *et al.*,

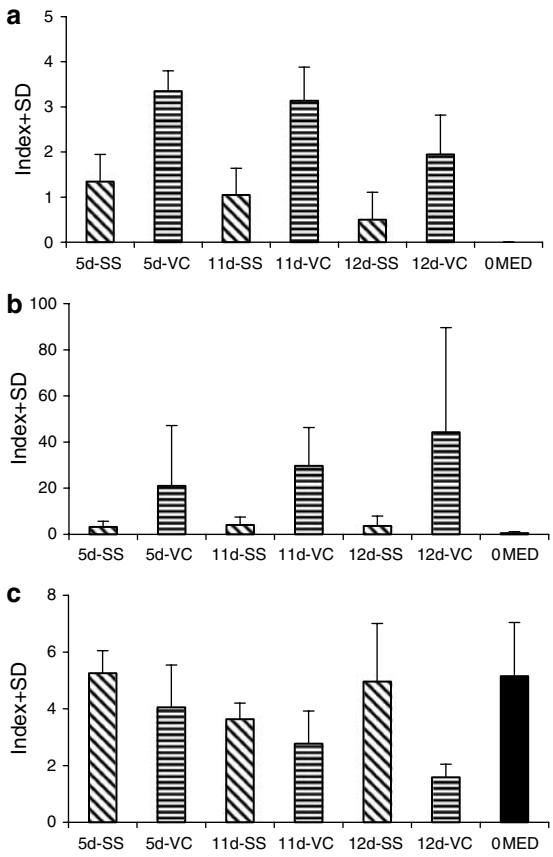

Figure 2. Daily sunscreen use abrogates DNA and immunological damage. Sunscreen prevents the epidermal effects of repeated daily sub-erythemal SSR exposure on (**a**) the accumulation of TT where 50% of cells reached a given scoring index (see Supplementary Material); very similar results were observed for an assessment of 25% of cells, (**b**) the accumulated expression of normal p53 protein, (**c**) the gradual time-dependent loss of LC in the vehicle control group. SS = sunscreen; VC = vehicle control.

2005). Comparisons at all three time points showed a very significant (*P*-value generally <0.01) reduction of TT in sunscreen groups compared with their vehicle controls. In another study, a sun protection factor of 15 gave good protection against TT when applied before four daily doses of 2 MED SSR (Al Mahroos *et al.*, 2002). There is considerable evidence that the CPD, and the TT in particular, initiates erythema and our data support this. Furthermore, CPD are able to cause potentially carcinogenic mutations, which can be prevented by sunscreens in mice (Ananthaswamy *et al.*, 2002).

The cellular response to CPD is regulated by p53 with two main protective outcomes; DNA repair or apoptosis (SBC) (Melnikova and Ananthaswamy, 2005). p53 expression has recently been reported as a possible biomarker for actinic keratoses, which may progress to squamous cell carcinoma (Einspahr *et al.*, 2006). Transgenic mouse studies have shown that failure to repair DNA photodamage, in particular transcription-coupled repair, results in SBC formation (Brash *et al.*, 2001) and that normal p53 status results in optimal SBC response (Ziegler *et al.*, 1994). Furthermore, other studies show that the CPD is the putative lesion for SBC (Jans *et al.*, 2005). No treatment had any effect on constitutive Bcl2 expression. The control vehicle p53 data showed high SDs, especially on day 12, which was the consequence of differing individual time course, whereas p53 expression on all the sunscreen sites was very low. Significant ($P<0.01$) protection by the sunscreen was only achieved on day 11. SBC are normally seen in human epidermis about 24 hours after acute erythemal UVR (Sheehan and Young, 2002), but SBC were not observed in the vehicle control sites which is surprising given the presence of CPD and p53 expression (Washio *et al.*, 1999; Sheehan and Young, 2002). However, mouse studies have shown that chronic irradiation (three times per week) results in a loss of the apoptotic response within 7 days which is associated with the loss of the Fas-ligand, which interacts with Fas (CD95) to induce apoptosis (Ouhtit *et al.*, 2000). Single exposure studies on human buttock skin have shown that UVB downregulates Fas-ligand and the receptor for tumor necrosis factor-related apoptosis-inducing ligand (Bachmann *et al.*, 2001). UVR-induced resistance to apoptosis may serve to maintain a viable and proliferating epidermis.

The depletion of antigen-presenting LC, along with the influx of $CD11b^+$ macrophages, is important in the immunomodulatory effects of UVR (Meunier *et al.*, 1995). The vehicle control data show a time-dependent depletion of LC that was prevented by the sunscreen.

The MIB1 data suggest that all treatments stimulated some epidermal proliferation, although the large interpersonal variation meant no treatment was significantly different from any other or the 0 MED control. This was supported by the epidermal layer data.

The sunscreen sites received an estimated $\sim$0.1 MED/day that resulted in some accumulation of CPD. Thus, avoiding sunburn to reduce skin cancer risk does not necessarily prevent DNA photodamage and indicates the importance of CPD repair in the prevention of skin cancer especially in the absence of an apoptotic response after repeated SSR exposure. The minimization of skin cancer risk in susceptible populations, especially where some degree of solar exposure is unavoidable, may ultimately require the incorporation of DNA repair enhancement technology in sunscreens (Yarosh *et al.*, 2005).

In conclusion, daily sub-erythemal SSR exposure in skin types I/II results in clinical, cellular, and molecular damage. Much of this damage, and in some cases all of it, can be inhibited by

a low sun protection factor sunscreen that may be effective in the prevention of long-term photodamage, including skin cancer. However, a preventive role for sunscreens has yet to be confirmed (IARC, 2001). Possible reasons for this include (i) insufficient application (Bech-Thomsen and Wulf, 1992; Diffey, 1996), (ii) inadequate immunoprotection (Kelly *et al.*, 2003) associated with poor UVA protection (Fourtanier *et al.*, 2005), (iii) probable greater use of sunscreens by high-risk groups, and (iv) their use/evaluation too late in life to be prophylactically effective.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary Text. Materials and Methods.

Figure S1. Molecular structures and absorption spectra of individual and sum of the individual spectra combined sunscreens determined with a UV spectrometer 8453 (Agilent Technologies, AG, Basel, Switzerland) using 3.3 mg polysilicone-15 and 1.1 mg butyl methoxydibenzoylmethane in 100 ml EtOH, respectively, that matches the ratio of 3:1 in the test formulation.

Table S1. Demographics and just perceptible MEDs of study volunteers.

Table S2. Antibody details and staining summary.

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